# LZI Methadone Metabolite (EDDP) Enzyme Immunoassay

IVD For In Vitro Diagnostic Use Only

REF 0190b (100/37.5 mL $R_1/R_2$ Kit)
0191b (1000/375 mL $R_1/R_2$ Kit)

2°C 8°C

***Lin-Zhi International, Inc.*** ***Outside USA Only***

## Intended Use

The Lin-Zhi International, Inc. (LZI) Methadone Metabolite (EDDP) Enzyme Immunoassay is an *in vitro* diagnostic test intended for the qualitative and semi-quantitative determination of methadone metabolite in human urine at a cutoff value of 100 ng/mL and 300 ng/mL when calibrated against methadone metabolite. The assay is designed for screening with the Beckman Coulter AU480 clinical chemistry analyzer.

The semi-quantitative mode is for purposes of (1) enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as gas or liquid chromatography/mass spectrometry (GC/MS or LC/MS) or (2) permitting laboratories to establish quality control procedures.

**The assay provides only a preliminary analytical result. A more specific alternative analytical chemistry method must be used in order to obtain a confirmed analytical result. Gas or liquid chromatography/mass spectrometry (GC/MS or LC/MS) is the preferred confirmatory method (1, 2). Clinical consideration and professional judgment should be exercised with any drug of abuse test result, particularly when the preliminary test result is positive.**

## Summary and Explanation of Test

Methadone is a synthetic diphenylheptanonylamine opioid that has similar analgesic activity and potency as morphine when administered parenterally. However, unlike morphine, it reliably retains its effectiveness when given orally, and tolerance and physical dependency develop slowly (3, 4).

Although methadone is prescribed to relieve chronic pain, its primary, medical application, however, is the detoxification and/or maintenance treatment of narcotic or heroin addiction (3, 5, 6). The abuse potential of methadone is comparable to that of morphine due to its similar pharmacological activity (3, 5, 7).

Methadone is readily absorbed from the gastrointestinal tract when ingested, and metabolized extensively in the liver. Initial N-demethylation results in normethadone metabolite, which rapidly undergoes cyclization followed by dehydration to form its major metabolite, 2-ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrolidine, commonly known as EDDP. Further N-demethylation yields a secondary metabolite, 2-ethyl-5-methyl-3, 3-diphenyl-1-pyrroline (EMDP). The metabolites are secreted in urine or bile along with unchanged drug (8).

## Assay Principle

The LZI Methadone Metabolite Enzyme Immunoassay is a homogeneous enzyme immunoassay ready-to-use liquid reagent. The assay is based on competition between EDDP in the sample and EDDP labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for a fixed amount of antibody in the reagent (9). Enzyme activity decreases upon binding to the antibody, and the drug concentration in the sample is measured in terms of enzyme activity.

In the absence of EDDP in the sample, EDDP-labeled G6PDH conjugate is bound to antibody, and the enzyme activity is inhibited. On the other hand, when EDDP is present in the sample, antibody binds to the free EDDP; the unbound G6PDH labeled with EDDP then exhibits its maximal enzyme activity. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH, resulting in an absorbance change that can be measured spectrophotometrically at a 340 nm primary wavelength.

## Reagents Provided

Antibody/Substrate Reagent ($R_1$): Contains mouse monoclonal anti-EDDP antibody, glucose-6-phosphate (G6P), nicotinamide adenine dinucleotide (NAD), stabilizers, and sodium azide (0.09%) as a preservative.

Enzyme-drug Conjugate Reagent ($R_2$): Contains glucose-6-phosphate dehydrogenase (G6PDH) labeled with EDDP in buffer with sodium azide (0.09%) as a preservative.

## Calibrators and Controls*

Materials required (but not provided)

**Calibrators and Controls are sold separately and contain negative human urine with sodium azide as a preservative.*

| METHADONE METABOLITE (EDDP) 100 ng/mL Calibrators | REF |
|---|---|
| Negative Calibrator | 0001 |
| Low Calibrator: Contains 50 ng/mL methadone metabolite | 0202b |
| Cutoff Calibrator: Contains 100 ng/mL methadone metabolite | 0203b |
| Intermediate Calibrator: Contains 250 ng/mL methadone metabolite | 0204b |
| High Calibrator: Contains 500 ng/mL methadone metabolite | 0205b |

| METHADONE METABOLITE (EDDP) 100 ng/mL Controls | REF |
|---|---|
| Level 1 Control: Contains 75 ng/mL methadone metabolite | 0207b |
| Level 2 Control: Contains 125 ng/mL methadone metabolite | 0208b |

| METHADONE METABOLITE (EDDP) 300 ng/mL Calibrators | REF |
|---|---|
| Negative Calibrator | 0001 |
| Low Calibrator: Contains 150 ng/mL methadone metabolite | 0192b |
| Cutoff Calibrator: Contains 300 ng/mL methadone metabolite | 0193b |
| Intermediate Calibrator: Contains 600 ng/mL methadone metabolite | 0194b |
| High Calibrator: Contains 1000 ng/mL methadone metabolite | 0195b |

| METHADONE METABOLITE (EDDP) 300 ng/mL Controls | REF |
|---|---|
| Level 1 Control: Contains 225 ng/mL methadone metabolite | 0197b |
| Level 2 Control: Contains 375 ng/mL methadone metabolite | 0198b |

## *Precautions and Warning*

- *This test is for in vitro diagnostic use only. Harmful if swallowed.*
- *Reagent contains sodium azide as a preservative, which may form explosive compounds in metal drain lines. When disposing such reagents or wastes, always flush with a large volume of water to prevent azide build-up. See National Institute for Occupational Safety and Health Bulletin: Explosive Azide Hazards (10).*
- *Do not use the reagents beyond their expiration dates.*

## Reagent Preparation and Storage

The reagents are ready-to-use. No reagent preparation is required. All assay components should be refrigerated at 2-8ºC when not in use. See the expiration date on individual bottle labels.

## Specimen Collection and Handling

Urine samples may be collected in plastic or glass containers. Some plastics may absorb drugs. Use of plastics such as polyethylene is recommended (11). Use fresh urine specimens for the test. If the sample cannot be analyzed immediately, it may be refrigerated at 2-8ºC for up to seven days. For longer storage, keep sample frozen at -20ºC and then thaw before use. Studies have shown EDDP analytes in urine are stable at -20ºC up to six months (12). Samples should be at room temperature (18-25ºC) for testing. Samples with high turbidity should be centrifuged before analysis.

Adulteration may cause erroneous results. If sample adulteration is suspected, obtain a new sample and forward both samples to the laboratory for testing.

*Handle all urine specimens as if they are potentially infectious.*

## Instrument

Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting sample, mixing reagents, measuring enzyme rates at 340 nm and timing the reaction accurately can be used to perform this homogeneous immunoassay.

Performance characteristics presented in this package insert have been validated on the Beckman Coulter AU480.

## Assay Procedure

Please refer to the specific parameters used for each analyzer before performing the assay.

**For 100 ng/mL Cutoff:** Typical assay parameters used for the Beckman Coulter AU480 analyzer include a 8 µL sample, 120 µL of antibody reagent ($R_1$), 45 µL of enzyme conjugate reagent ($R_2$), 10 µL dilution following addition of $R_2$ at 37ºC incubation temperature, 12-16 reading frame, FIXED method, and 340 nm primary wavelength.

For qualitative analysis use the 100 ng/mL as the cutoff calibrator. For semi-quantitative analysis, use all five calibrators. Recalibration should be performed after reagent bottle change or a change in calibrators or reagent lot. Two levels of controls are also available for monitoring the cutoff level: 75 ng/mL and 125 ng/mL.

## Assay Procedure, continued

**For 300 ng/mL Cutoff:** Typical assay parameters used for the Beckman Coulter AU480 analyzer include a 4 µL sample, 120 µL of antibody reagent ($R_1$), 45 µL of enzyme conjugate reagent ($R_2$), 10 µL dilution following addition of $R_2$ at 37ºC incubation temperature, 12-16 reading frame, FIXED method, and 340 nm primary wavelength.

For qualitative analysis use the 300 ng/mL as the cutoff calibrator. For semi-quantitative analysis, use all five calibrators. Recalibration should be performed after reagent bottle change or a change in calibrators or reagent lot. Two levels of controls are also available for monitoring the cutoff level: 225 ng/mL and 375 ng/mL.

## Calibration and Quality Control

Good laboratory practices recommend the use of at least two levels of control specimens (one positive and one negative control near the cutoff) to ensure proper assay performance. Controls should be run with each new calibration and after specific maintenance or troubleshooting procedures as detailed in the instrument system manual. Each laboratory should establish its own control frequency. If any trends or sudden change in control value are observed, review all operating parameters, or contact LZI technical support for further assistance. Laboratories should comply with all federal, state, and local laws, as well as all guidelines and regulations.

## Results

**Note:** A preliminary positive test result does not necessarily mean a person took illegal drugs, and a negative test result does not necessarily mean a person did not take illegal drugs. There are a number of factors that influence the reliability of drug tests.
Normal populations have a value below the cutoff. Affected populations have a value above the cutoff.

**Qualitative:** The cutoff calibrator, which contains either 100 ng/mL or 300 ng/mL of methadone metabolite (EDDP), is used as a reference for distinguishing a preliminary positive from negative samples. Samples producing a positive or "0" absorbance value are considered preliminary positive. Samples producing a negative absorbance value are considered negative. Results of this assay distinguish preliminary positive from negative samples only. The amount of drug detected in a preliminary positive sample cannot be estimated.

**Semi-Quantitative**: The semi-quantitative mode is for purposes of (1) enabling laboratories to determine an appropriate dilution of the specimen for confirmation by a confirmatory method such as GC/MS or LC/MS or (2) permitting laboratories to establish quality control procedures.
When an approximation of concentration is required, a calibration curve can be established with five calibrators. The concentration of methadone metabolite in the sample may then be estimated from the calibration curve. Results equal to or greater than the respective cutoff value are considered preliminary positive. Concentration values below the respective cutoff indicate a negative result.

## Limitations

1. *A preliminary positive result from the assay indicates only the presence of methadone metabolite. The test is not intended for quantifying this single analyte in samples.*
2. *A preliminary positive result does not necessarily indicate drug abuse.*
3. *A negative result does not necessarily mean a person did not take illegal drugs.*
4. *Care should be taken when reporting results as numerous factors (e.g., fluid intake, endogenous or exogenous interferents) may influence the urine test result.*
5. *Preliminary positive results should be confirmed by other affirmative, analytical chemistry methods (e.g., chromatography), preferably GC/MS or LC/MS.*
6. *The test is designed for use with human urine only.*
7. *The test is not for therapeutic drug monitoring.*

## Typical Performance Characteristics

The results shown below were performed with a single Beckman Coulter AU480 automated chemistry analyzer.

**Precision for 100 ng/mL Cutoff:**
Semi-quantitative analysis: The following concentrations were determined with reference curves from five calibrators. Typical results (ng/mL) are as follows:

| 100 ng/mL Cutoff Result: | | Within Run (N=22) | | Total Precision (N=88) | |
|---|---|---|---|---|---|
| **Concentration** | **% of Cutoff** | **# Samples** | **EIA Result** | **# Samples** | **EIA Result** |
| 0 ng/mL | 0% | 22 | 22 Neg | 88 | 88 Neg |
| 25 ng/mL | 25% | 22 | 22 Neg | 88 | 88 Neg |
| 50 ng/mL | 50% | 22 | 22 Neg | 88 | 88 Neg |
| 75 ng/mL | 75% | 22 | 22 Neg | 88 | 88 Neg |
| 100 ng/mL | 100% | 22 | 11 Neg/ 11 Pos | 88 | 48 Neg/ 40 Pos |
| 125 ng/mL | 125% | 22 | 22 Pos | 88 | 88 Pos |
| 150 ng/mL | 150% | 22 | 22 Pos | 88 | 88 Pos |
| 175 ng/mL | 175% | 22 | 22 Pos | 88 | 88 Pos |
| 200 ng/mL | 200% | 22 | 22 Pos | 88 | 88 Pos |

Qualitative analysis: The following concentrations were evaluated. Typical qualitative results (measured by ΔOD, mAU) are as follows:

| 100 ng/mL Cutoff Result: | | Within Run (N=22) | | Total Precision (N=88) | |
|---|---|---|---|---|---|
| **Concentration** | **% of Cutoff** | **# Samples** | **EIA Result** | **# Samples** | **EIA Result** |
| 0 ng/mL | 0% | 22 | 22 Neg | 88 | 88 Neg |
| 25 ng/mL | 25% | 22 | 22 Neg | 88 | 88 Neg |
| 50 ng/mL | 50% | 22 | 22 Neg | 88 | 88 Neg |
| 75 ng/mL | 75% | 22 | 22 Neg | 88 | 88 Neg |
| 100 ng/mL | 100% | 22 | 13 Neg/ 9 Pos | 88 | 54 Neg/ 34 Pos |
| 125 ng/mL | 125% | 22 | 22 Pos | 88 | 88 Pos |
| 150 ng/mL | 150% | 22 | 22 Pos | 88 | 88 Pos |
| 175 ng/mL | 175% | 22 | 22 Pos | 88 | 88 Pos |
| 200 ng/mL | 200% | 22 | 22 Pos | 88 | 88 Pos |

**Precision for 300 ng/mL Cutoff:**
Semi-quantitative analysis: The following concentrations were determined with reference curves from five calibrators. Typical results (ng/mL) are as follows:

| 300 ng/mL Cutoff Result: | | Within Run (N=22) | | Total Precision (N=88) | |
|---|---|---|---|---|---|
| **Concentration** | **% of Cutoff** | **# Samples** | **EIA Result** | **# Samples** | **EIA Result** |
| 0 ng/mL | 0% | 22 | 22 Neg | 88 | 88 Neg |
| 75 ng/mL | 25% | 22 | 22 Neg | 88 | 88 Neg |
| 150 ng/mL | 50% | 22 | 22 Neg | 88 | 88 Neg |
| 225 ng/mL | 75% | 22 | 22 Neg | 88 | 88 Neg |
| 300 ng/mL | 100% | 22 | 6 Neg/ 16 Pos | 88 | 36 Neg/ 52 Pos |
| 375 ng/mL | 125% | 22 | 22 Pos | 88 | 88 Pos |
| 450 ng/mL | 150% | 22 | 22 Pos | 88 | 88 Pos |
| 525 ng/mL | 175% | 22 | 22 Pos | 88 | 88 Pos |
| 600 ng/mL | 200% | 22 | 22 Pos | 88 | 88 Pos |

Qualitative analysis: The following concentrations were evaluated. Typical qualitative results (measured by ΔOD, mAU) are as follows:

| 300 ng/mL Cutoff Result: | | Within Run (N=22) | | Total Precision (N=88) | |
|---|---|---|---|---|---|
| **Concentration** | **% of Cutoff** | **# Samples** | **EIA Result** | **# Samples** | **EIA Result** |
| 0 ng/mL | 0% | 22 | 22 Neg | 88 | 88 Neg |
| 75 ng/mL | 25% | 22 | 22 Neg | 88 | 88 Neg |
| 150 ng/mL | 50% | 22 | 22 Neg | 88 | 88 Neg |
| 225 ng/mL | 75% | 22 | 22 Neg | 88 | 88 Neg |
| 300 ng/mL | 100% | 22 | 7 Neg/ 15 Pos | 88 | 33 Neg/ 55 Pos |
| 375 ng/mL | 125% | 22 | 22 Pos | 88 | 88 Pos |
| 450 ng/mL | 150% | 22 | 22 Pos | 88 | 88 Pos |
| 525 ng/mL | 175% | 22 | 22 Pos | 88 | 88 Pos |
| 600 ng/mL | 200% | 22 | 22 Pos | 88 | 88 Pos |

**Accuracy for 100 ng/mL Cutoff:** Eighty-seven **(**87) unaltered clinical urine specimens were tested with LZI Methadone Metabolite (EDDP) Enzyme Immunoassay and confirmed with LC/MS. Specimens having a methadone metabolite concentration greater than 100 ng/mL by LC/MS are defined as positive, and specimens with concentrations lower than 100 ng/mL by LC/MS are defined as negative in the table below. The correlation results are summarized as follows (near cutoff samples are defined as ±50% of the cutoff value):
Qualitative Accuracy Study:

| 100 ng/mL Cutoff | Neg | < 50% below the cutoff | Near Cutoff Neg | Near Cutoff Pos | > 50% above the cutoff |
|---|---|---|---|---|---|
| Positive | 0 | 0 | 0 | 2 | 40 |
| Negative | 23 | 11 | 9 | 2* | 0 |

The following table summarizes the result for the two discrepant samples:

| Sample # | Assay Result: | | LC/MS (ng/mL) |
|---|---|---|---|
| | LC/MS | LZI EIA | |
| Sample #45* | + | - | 103.1 |
| Sample #46* | + | - | 126.0 |

*Values are discrepant between the cutoff and 50% above the cutoff concentration (100 – 149.9 ng/mL)
Semi-Quantitative Accuracy Study:

| 100 ng/mL Cutoff | Neg | < 50% below the cutoff | Near Cutoff Neg | Near Cutoff Pos | > 50% above the cutoff |
|---|---|---|---|---|---|
| Positive | 0 | 0 | 0 | 2 | 40 |
| Negative | 23 | 11 | 9 | 2* | 0 |

The following table summarizes the result for the two discrepant samples:

| Sample # | Assay Result: | | LC/MS (ng/mL) |
|---|---|---|---|
| | LC/MS | LZI EIA | |
| Sample #45* | + | - | 103.1 |
| Sample #46* | + | - | 126.0 |

*Values are discrepant between the cutoff and 50% above the cutoff concentration (100 – 149.9 ng/mL)

**Accuracy for 300 ng/mL Cutoff:** Eighty-four (84) unaltered clinical urine specimens were tested with LZI Methadone Metabolite (EDDP) Enzyme Immunoassay and confirmed with LC/MS. Specimens having a methadone metabolite concentration greater than 300 ng/mL by LC/MS are defined as positive, and specimens with concentrations lower than 300 ng/mL by LC/MS are defined as negative in the table below. The correlation results are summarized as follows (near cutoff samples are defined as ±50% of the cutoff value):

Qualitative Accuracy Study:

| 300 ng/mL Cutoff | Neg | <50% below the cutoff | Near Cutoff Neg | Near Cutoff Pos | >50% above the cutoff |
|---|---|---|---|---|---|
| Positive | 0 | 0 | 0 | 4 | 38 |
| Negative | 21 | 15 | 6 | 0 | 0 |

Semi-Quantitative Accuracy Study:

| 300 ng/mL Cutoff | Neg | <50% below the cutoff | Near Cutoff Neg | Near Cutoff Pos | >50% above the cutoff |
|---|---|---|---|---|---|
| Positive | 0 | 0 | 0 | 4 | 38 |
| Negative | 21 | 15 | 6 | 0 | 0 |

**Analytical Recovery for 100 ng/mL Cutoff:** To demonstrate linearity for purposes of sample dilution and quality control, a drug-free pool of urine was spiked with methadone metabolite to 500 ng/mL and was subsequently diluted. Each sample was run in ten replicates and the average was used determine percent recovery compared to the expected target value. Results are listed in the table below:

| % Dilution | Expected Value (ng/mL) | Observed Value (ng/mL) | % Recovery |
|---|---|---|---|
| 100% | 0 | -3.1 | N/A |
| 98% | 10 | 5.2 | 51.7% |
| 90% | 50 | 49.0 | 97.9% |
| 80% | 100 | 95.8 | 95.8% |
| 75% | 150 | 153.7 | 102.5% |
| 60% | 200 | 203.0 | 101.5% |
| 50% | 250 | 245.8 | 98.3% |
| 40% | 300 | 312.7 | 104.2% |
| 30% | 350 | 369.1 | 105.5% |
| 20% | 400 | 412.9 | 103.2% |
| 10% | 450 | 443.0 | 98.4% |
| 0% | 500 | 478.2 | 95.6% |

**Analytical Recovery for 300 ng/mL Cutoff:** To demonstrate linearity for purposes of sample dilution and quality control, a drug-free pool of urine was spiked with methadone metabolite to 1000 ng/mL and was subsequently diluted. Each sample was run in ten replicates and the average was used determine percent recovery compared to the expected target value. Results are listed in the table below:

| % Dilution | Expected Value (ng/mL) | Observed Value (ng/mL) | % Recovery |
|---|---|---|---|
| 100% | 0 | -6.4 | N/A |
| 98% | 20 | 5.8 | 29.0% |
| 90% | 100 | 83.1 | 83.1% |
| 80% | 200 | 196.2 | 98.1% |
| 75% | 300 | 295.9 | 98.6% |
| 60% | 400 | 422.5 | 105.6% |
| 50% | 500 | 519.0 | 103.8% |
| 40% | 600 | 595.3 | 99.2% |
| 30% | 700 | 709.4 | 101.3% |
| 20% | 800 | 807.1 | 100.9% |
| 10% | 900 | 878.4 | 97.6% |
| 0% | 1000 | 940.9 | 94.1% |

**Specificity:** Various potentially interfering substances were tested for cross-reactivity with the assay. Test compounds were spiked into the drug-free urine calibrator matrix individually to various concentrations and evaluated against the cutoff calibrator. The table listed below shows the concentration of each test compound that gave a response approximately equivalent to that of the cutoff calibrator or the maximal concentration of the compound tested that did not exhibit interference.

**Methadone Metabolite and Structurally Related Compounds for 100 ng/mL Cutoff:**

| Compound | Spiked [ ] (ng/mL) | EIA [ ] (ng/mL) | % Cross-Reactivity |
|---|---|---|---|
| EDDP | 100 | Pos | 100% |
| EMDP | 100000 | Neg | <0.1% |
| Methadone | 300000 | Neg | <0.1% |
| LAAM HCl | 500000 | Neg | <0.1% |
| (±)-α-Methadol | 300000 | Neg | <0.1% |
| (-)-Isomethadone HCl | 60000 | Neg | <0.2% |
| (-)-α-Noracetylmethadol (Nor-LAAM) HCl | 300000 | Neg | <0.1% |

**Structurally Unrelated Pharmacological Compounds for 100 ng/mL Cutoff:**

The table below lists the maximal concentration of the compound tested without interference.

| Interfering Substances | Spiked [ ] (ng/mL) |
|---|---|
| Acetaminophen | 100000 |
| 6-Acetylmorphine | 10000 |
| Acetylsalicylic Acid | 100000 |
| Alimemazine | 1000 |
| Amitriptyline | 100000 |
| Amlodipine | 100000 |
| Amoxicillin | 100000 |
| *d*-Amphetamine | 100000 |
| Atorvastatin | 20000 |
| Benzoylecgonine | 100000 |
| Buprenorphine | 15000 |
| Bupropion | 100000 |
| Caffeine | 100000 |
| Carbamazepine | 100000 |
| Cetirizine | 100000 |
| Chlorpheniramine | 100000 |
| Chlorpromazine | 50000 |
| Clomipramine | 100000 |
| Codeine | 100000 |
| Cyamemazine | 12000 |
| Desipramine | 100000 |
| Diphenhydramine | 100000 |
| Doxylamine | 100000 |
| Duloxetine | 20000 |
| Fentanyl | 10000 |
| Fluoxetine | 100000 |
| Fluphenazine | 100000 |
| Gabapentin | 100000 |
| Hydrocodone | 100000 |
| Hydromorphone | 100000 |
| Ibuprofen | 100000 |
| Imipramine | 100000 |
| Levomepromazine | 40000 |
| Lisinopril | 100000 |
| Losartan | 10000 |
| Loratadine | 100000 |
| MDA (3,4-Methylene-Dioxyamphetamine) | 100000 |
| MDEA | 100000 |
| MDMA (3,4-Methylene-Dioxymethylamphetamine) | 100000 |
| Meperidine | 100000 |
| Metformin | 100000 |
| Methylphenidate | 100000 |
| Metoprolol | 100000 |
| *d*-Methamphetamine | 100000 |
| Methapyrilene | 10000 |
| Methaqualone | 100000 |
| Metronidazole | 100000 |
| Morphine | 100000 |
| Nicotine | 100000 |
| Nortriptyline | 100000 |
| Omeprazole | 100000 |
| Oxazepam | 100000 |
| Oxycodone | 100000 |
| Oxymorphone | 100000 |
| Phencyclidine | 10000 |
| Phenobarbital | 100000 |
| Promethazine | 5000 |
| (1S,2S)-(+) Pseudoephedrine | 100000 |
| Quetiapine | 100000 |
| Ranitidine | 100000 |
| Salbutamol (Albuterol) | 100000 |
| Sertraline | 5000 |
| THC-COOH (11-Nor-Delta-9-THC-9-Carboxylic Acid) | 1000 |
| Thioridazine | 20000 |
| l-Thyroxine | 10000 |
| Tramadol | 100000 |
| Verapamil | 100000 |
| Zolpidem | 10000 |

*It is possible that other substances and/or factors not listed above may interfere with the test and cause false positive results.*

**Methadone Metabolite and Structurally Related Compounds for 300 ng/mL Cutoff:**

| Compound | Spiked [ ] (ng/mL) | EIA [ ] (ng/mL) | % Cross-Reactivity |
|---|---|---|---|
| EDDP | 300 | Pos | 100% |
| EMDP | 100000 | Neg | <0.1% |
| Methadone | 500000 | Neg | <0.1% |
| LAAM HCl | 500000 | Neg | <0.1% |
| (±)-α-Methadol | 300000 | Neg | <0.1% |
| (-)-Isomethadone HCl | 200000 | Neg | <0.1% |
| (-)-α-Noracetylmethadol (Nor-LAAM) HCl | 300000 | Neg | <0.1% |

**Structurally Unrelated Pharmacological Compounds for 300 ng/mL Cutoff:**

The table listed below shows the maximal concentration of the compound tested without interference.

| Interfering Substances | Spiked [ ] (ng/mL) |
|---|---|
| Acetaminophen | 100000 |
| 6-Acetylmorphine | 10000 |
| Acetylsalicylic Acid | 100000 |
| Alimemazine | 4000 |
| Amitriptyline | 100000 |
| Amlodipine | 100000 |
| Amoxicillin | 100000 |
| *d*-Amphetamine | 100000 |
| Atorvastatin | 20000 |
| Benzoylecgonine | 100000 |
| Buprenorphine | 15000 |
| Bupropion | 100000 |
| Caffeine | 100000 |
| Carbamazepine | 100000 |
| Cetirizine | 100000 |
| Chlorpheniramine | 100000 |
| Chlorpromazine | 100000 |
| Clomipramine | 100000 |
| Codeine | 100000 |
| Cyamemazine | 25000 |
| Desipramine | 100000 |
| Diphenhydramine | 100000 |
| Doxylamine | 100000 |
| Duloxetine | 60000 |
| Fentanyl | 10000 |
| Fluoxetine | 100000 |
| Fluphenazine | 100000 |
| Gabapentin | 100000 |
| Hydrocodone | 100000 |
| Hydromorphone | 100000 |
| Ibuprofen | 100000 |
| Imipramine | 100000 |
| Levomepromazine | 100000 |
| Lisinopril | 100000 |
| Losartan | 10000 |
| Loratadine | 100000 |
| MDA (3,4-Methylene-Dioxyamphetamine) | 100000 |
| MDEA | 100000 |
| MDMA (3,4-Methylene-Dioxymethylamphetamine) | 100000 |
| Meperidine | 100000 |
| Metformin | 100000 |
| Methylphenidate | 100000 |
| Metoprolol | 100000 |
| d-Methamphetamine | 100000 |
| Methapyrilene | 40000 |
| Methaqualone | 100000 |
| Metronidazole | 100000 |
| Morphine | 100000 |
| Nicotine | 100000 |
| Nortriptyline | 100000 |
| Omeprazole | 100000 |
| Oxazepam | 100000 |
| Oxycodone | 100000 |
| Oxymorphone | 100000 |
| Phencyclidine | 20000 |
| Phenobarbital | 100000 |
| Promethazine | 15000 |
| (1S,2S)-(+)Pseudoephedrine | 100000 |
| Quetiapine | 100000 |
| Ranitidine | 100000 |
| Salbutamol (Albuterol) | 100000 |
| Sertraline | 15000 |

**Structurally Unrelated Pharmacological Compounds for 300 ng/mL Cutoff, continued:**

| Interfering Substances | Spiked [ ] (ng/mL) |
|---|---|
| THC-COOH (11-Nor-Delta-9-THC-9-Carboxylic Acid) | 1000 |
| Thioridazine | 90000 |
| l-Thyroxine | 10000 |
| Tramadol | 100000 |
| Verapamil | 100000 |
| Zolpidem | 10000 |

*It is possible that other substances and/or factors not listed above may interfere with the test and cause false positive results.*

**Interference: Endogenous Substances for 100 ng/mL Cutoff**

The following potentially interfering compounds were spiked into a pool of processed drug-free urine to the desired concentrations and then EDDP was spiked to a final concentration of 0 ng/mL or the negative control concentration of 75 ng/mL, or the positive control concentration of 125 ng/mL. The spiked solution is evaluated against the assay's calibration curve. Results indicate there is no major interference with these compounds at physiological relevant concentrations as all spiked samples gave correct responding preliminary positive/negative results against the cutoff value of 100 ng/mL. The table listed below shows the maximal concentration of the compound tested without interference.

| Interfering Substances | Spiked [ ] (mg/dL) |
|---|---|
| Acetone | 1000 |
| Ascorbic Acid | 1500 |
| Creatinine | 500 |
| Ethanol | 1000 |
| Galactose | 10 |
| γ-Globulin | 500 |
| Glucose | 3000 |
| Hemoglobin | 300 |
| Human Serum Albumin | 500 |
| Oxalic Acid | 100 |
| Riboflavin | 7.5 |
| Urea | 6000 |
| Sodium Chloride | 4000 |
| pH 3 | N/A |
| pH 4 | N/A |
| pH 5 | N/A |
| pH 6 | N/A |
| pH 7 | N/A |
| pH 8 | N/A |
| pH 9 | N/A |
| pH 10 | N/A |
| pH 11 | N/A |

**Interference: Endogenous Substances for 300 ng/mL Cutoff**

The following potentially interfering compounds were spiked into a pool of processed drug-free urine to the desired concentrations and then EDDP was spiked to a final concentration of 0 ng/mL or the negative control concentration of 225 ng/mL, or the positive control concentration of 375 ng/mL. The spiked solution is evaluated against the assay's calibration curve. Results indicate there is no major interference with these compounds at physiological relevant concentrations as all spiked samples gave correct responding preliminary positive/negative results against the cutoff value of 300 ng/mL. The table listed below shows the maximal concentration of the compound tested without interference.

| Interfering Substances | Spiked [ ] (mg/dL) |
|---|---|
| Acetone | 1000 |
| Ascorbic Acid | 1500 |
| Creatinine | 500 |
| Ethanol | 1000 |
| Galactose | 10 |
| γ-Globulin | 500 |
| Glucose | 3000 |
| Hemoglobin | 300 |
| Human Serum Albumin | 500 |
| Oxalic Acid | 100 |
| Riboflavin | 7.5 |
| Urea | 6000 |
| Sodium Chloride | 6000 |
| pH 3 | N/A |
| pH 4 | N/A |
| pH 5 | N/A |
| pH 6 | N/A |

**Interference: Endogenous Substances for 300 ng/mL Cutoff, continued:**

| Interfering Substances | Spiked [ ] (mg/dL) |
|---|---|
| pH 7 | N/A |
| pH 8 | N/A |
| pH 9 | N/A |
| pH 10 | N/A |
| pH 11 | N/A |

**Specific Gravity for 100 ng/mL Cutoff:** Samples ranging in specific gravity from 1.004 to 1.025 were spiked with methadone metabolite to a final concentration of 0 ng/mL, the negative control concentration of 75 ng/mL, or the positive control concentration of 125 ng/mL. No interference was observed.

**Specific Gravity for 300 ng/mL Cutoff:** Samples ranging in specific gravity from 1.004 to 1.025 were spiked with methadone metabolite to a final concentration of 0 ng/mL, the negative control concentration of 225 ng/mL, or the positive control concentration of 375 ng/mL. No interference was observed.

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

## Symbols:

LZI uses the symbols and signs listed on the symbol glossary on the website. Visit www.lin-zhi.com/symbol-glossary for detailed information.

Additions, deletions, or changes are indicated by a change bar in the margin.
For technical assistance please call: (408) 970-8811


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